PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07D 221/18, 491/04, A61K 31/435 // (C07D 491/04, 317:00, 221:00)

A1

(11) International Publication Number: WO 98/12181

(43) International Publication Date: 26 March 1998 (26.03.98)

(21) International Application Number: PCT/US97/17012

(22) International Filing Date: 23 September 1997 (23.09.97)

(30) Priority Data:
60/026,511 23 September 1996 (23.09.96) US

(71) Applicant: RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY [US/US]; Old Queens Building, Somerset and George Streets, New Brunswick, NJ 08903 (US).

(71)(72) Applicants and Inventors: LAVOIE, Edmond, J. [US/US]; 3 Guilford Court, Princeton Junction, NJ 08550 (US). LIU, Leroy, Fong [US/US]; 5 Fairacres Drive, Bridgewater, NJ 08807 (US). MAKHEY, Darshan [IN/US]; 720 Bevier Road, Piscataway, NJ 08854 (US).

(74) Agent: VIKSNINS, Ann, S.; Schwegman, Lundberg, Woessner & Kluth, P.O. Box 2938, Minneapolis, MN 55402 (US).

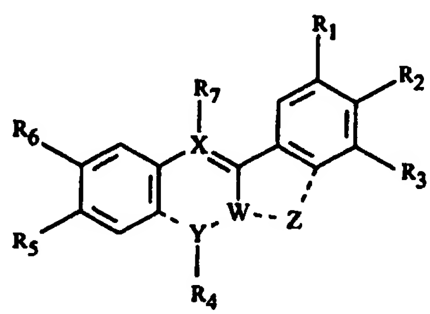
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: SUBSTITUTED HETEROCYCLES AS ANTI-TUMOR AGENTS

(57) Abstract

The present invention provides compounds of formula (I) wherein R_1-R_7, W, X, Y, and Z have any of the values defined in the specification, and pharmaceutically acceptable salt thereof, that are useful as anticancer agents. Also disclosed are pharmaceutical compositions comprising one or more compounds of formula (I), processes for preparing compounds of formula (I), and intermediates useful for preparing compounds of formula (I).



(I)

SUBSTITUTED HETEROCYLES AS ANTI-TUMOR AGENTS

Background of the Invention

DNA-topoisomerases are enzymes which are present in the nuclei of cells where they catalyze the breaking and rejoining of DNA strands, which control the topological state of DNA. Recent studies also suggest that topoisomerases are also involved in regulating template supercoiling during RNA transcription. There are two major classes of mammalian topoisomerases. DNA-topoisomerase-I catalyzes changes in the topological state of duplex DNA by performing transient single-strand breakage-union cycles. In contrast, mammalian topoisomerase II alters the topology of DNA by causing a transient enzyme bridged double-strand break, followed by strand passing and resealing. Mammalian topoisomerase II has been further classified as Type II α and Type II β. The antitumor activity associated with agents which are topoisomerase poisons is associated with their ability to stabilize the enzyme-DNA cleavable complex. This drug-induced stabilization of the enzyme-DNA cleavable complex effectively converts the enzyme into a cellular poison.

Several antitumor agents in clinical use have potent activity as mammalian topoisomerase II poisons. These include adriamycin, actinomycin D, daunomycin, VP-16, and VM-26. In contrast to the number of clinical and experimental drugs which act as topoisomerase II poisons, there are currently only a limited number of agents which have been identified as topoisomerase I poisons. Camptothecin and its structurally-related analogs are among the most extensively studied topoisomerase I poisons. Recently, bi- and terbenzimidazoles (Chen et al., *Cancer Res.* **1993**, *53*, 1332-1335; Sun et al., *J. Med. Chem.* **1995**, *38*, 3638-3644; Kim et al., *J. Med. Chem.* **1996**, *39*, 992-998), certain benzo[c]phenanthridine and protoberberine alkaloids and their synthetic analogs (Makhey et al., *Med. Chem. Res.* **1995**, *5*, 1-12; Janin et al., *J. Med. Chem* **1975**, *18*, 708-713; Makhey et al., *Bioorg. & Med. Chem.* **1996**, *4*, 781-791), as well as the fungal metabolites, bulgarcin (Fujii et al., *J. Biol. Chem.*

1993, *268*, 13160-13165) and saintopin (Yamashita et al., *Biochemistry* **1991**, *30*, 5838-5845) and indolocarbazoles (Yamashita et al., *Biochemistry* **1992**, *31*, 12069-12075) have been identified as topoisomerase I poisons.

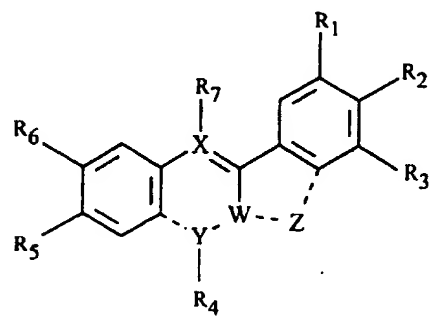
The exceptional topoisomerase poisoning observed with coralyne, nitidine, 5,6-dihydro-8-desmethylcoralyne and related analogs prompted several recent studies on those structural features which are associated with their ability to act specifically as poisons of topoisomerase I or topoisomerase II (Gatto et al., *Cancer Res.* **1996**, *56*, 2795-2800; Wang et al., *Chem. Res. Toxicol.* **1996**, *9*, 75-83; Wang et al., *Chem. Res. Toxicol.*, **1993**, *6*, 813-818). A common feature associated with all three of these agents is the presence of a 3-phenylisoquinolinium moiety within their structure.

Despite the observation that several of these compounds had similar potency to camptothecin as a topoisomerase I poison or similar potency to VM-26 as a topoisomerase II poison, they possessed only modest cytotoxic activity. The absence of a more direct correlation with their potency as topoisomerase poisons was attributed, in part, to the likelihood that these agents are not likely to be absorbed as effectively into cells as either camptothecin or VM-26. The presence of the quaternary ammonium group most likely impedes cellular uptake. It has been speculated that agents such as coralyne and nitidine may need to undergo hydrolysis to permit effective transport, with subsequent dehydration or cyclodehydration to reform the quaternary ammonium parent compound. This may explain the relatively poor antitumor activity observed *in vivo* with agents such as coralyne or nitidine.

It is clear that the need exists for anti-cancer agents with improved activity.

Summary of the Invention

The present invention provides compounds of formula (I):



(I)

wherein R_1, R_2, R_3, R_5 and R_6 are independently H, OH, NO_2, NH_2, halo, $NHCO(C_1-C_8)$alkyl or (C_1-C_8)alkoxy; R_1 and R_2 together are $-OCH_2O-$; R_2 and R_3 together are $-OCH_2O-$; or R_5 and R_6 together are $-OCH_2O-$;

R_4 and R_7 are independently H, (C_1-C_8)alkyl or absent;

W is C or N;

X is C or N;

Y is -C=, -N= or a direct bond, provided that where Y is -C=, X is N; and

Z is -CH=CH-, $-(CH_2)_2-$ or absent;

or a pharmaceutically acceptable salt thereof.

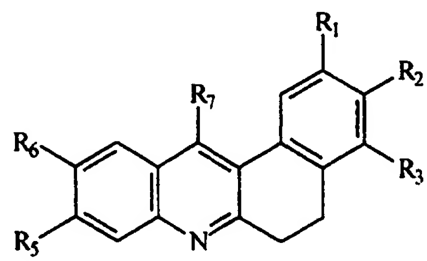
According to one preferred embodiment of the invention, W is N and Y is a direct bond. In another preferred embodiment, W is C and Y is -C= or -N=.

According to another preferred embodiment of the invention, Z is -CH=CH- or $-(CH_2)_2-$. In another preferred embodiment, R_5 and R_6 are each (C_1-C_8)alkoxy, preferably $-OCH_3$, or together are $-OCH_2O-$. Preferably R_3 is H. In a preferred embodiment, one or both of R_1 and R_2 is (C_1-C_8)alkoxy, preferably $-OCH_3$, or together are $-OCH_2O-$. In another prefered embodiment R_2 and R_3 together are $-OCH_2O-$.

Preferably, when X is C, R_7 is H, methyl, or ethyl; or when X is C, R_7 is H. Preferably, when X is N, R_7 is absent or CH_3. Similarly, it is preferred that

when Y is -C=, R_4 is H; and when Y is -N=, R_4 is absent or CH_3. It will be understood that when X or Y is substituted N, the N will have a positive charge.

A preferred group of compounds of formula I are compounds of formula II:



(II)

wherein R_1 R_2, R_3, R_5, R_6, and R_7 have any of the values or preferred values defined herein for a compound of formula I; or a pharmaceutically acceptable salt thereof.

The compounds of formula (I) have been shown to be effective cytotoxic agents against cancer cells, including drug-resistant cancer cells. Additionally, certain compounds of formula I show inhibitory activity against topoisomerase I. Accordingly, the invention also provides a method of inhibiting cancer cell growth *in vitro*, or *in vivo*, comprising administering to a mammal afflicted with cancer an amount of a compound of formula (I), effective to inhibit the growth of said cancer cells. According to the invention, the compound or its salt may be administered in combination with a pharmaceutically acceptable carrier.

The invention also provides pharmaceutical compositions comprising a compound of the invention in combination with a pharmaceutically acceptable carrier, as well as processes for preparing compounds of the invention, and novel intermediates useful for the synthesis of compounds of the invention.

Brief Descripti n of the Figures

Figure 1	illustrates the synthesis of representative benz[c]acridines of formula I;
Figure 2	illustrates the synthesis of representative benz[a]acridines of formula I;
Figure 3	illustrates the synthesis of representative compounds of the invention; and
Figure 4	illustrates the synthesis of intermediates useful for preparing compounds of formula I.

Detailed Description

According to the invention, cancer cells are inhibited *in vitro* or *in vivo*, by administration to a mammal afflicted with cancer of an effective amount of the compounds of formula (I). As used herein, an "effective amount" is that amount which results in an inhibition of growth of the target cancer cells. As described herein, a suitable dose will be in the range of about 0.5 to about 100 mg/kg of body weight per day.

The compounds and compositions described herein are believed to be effective in the treatment of solid mammalian tumors or hematologic malignancies. These solid tumors include cancers of the head and neck, lung, mesothelioma, mediastinum, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, rectum, anus, kidney, ureter, bladder, prostate, urethra, penis, testis, gynecological organs, ovarian, breast, endocrine system, skin central nervous system; sarcomas of the soft tissue and bone; and melanoma of cutaneous and intraocular origin. Hematological malignancies include childhood leukemia and lymphomas, Hodgkin's disease, lymphomas of lymphocytic and cutaneous origin, acute and chronic leukemia, plasma cell neoplasm and cancers associated with AIDS. The preferred mammalian species for treatment are humans and domesticated animals.

Benz[c]acridines of formula I (X is N and Y is -C=), may conveniently be prepared by the route illustrated in Figure 1. Reaction of 2-amino-4,5-

dimethoxyacetophenone, **4**, with 6,7-dimethoxy-1-tetralone, **5**, provided 5,6-dihydro-2,3,9,10-tetramethoxybenz[c]acridine, **6**, which, when heated as a suspension in decalin at 190°C in the presence of Pd/C, could be converted to 2,3,9,10-tetramethoxybenz[c]acridine, 7. Both **6** and **7** could be converted to their 12-methyl derivatives by reaction with dimethyl sulfate to form the quaternary ammonium salts, **8** and **9**, respectively.

Benz[a]acridine analogs (X is C and Y is -N=) may conveniently be prepared using procedures similar to those illustrated in Figure 2. Knoevenagel condensation of the appropriate *o*-nitrobenzaldehyde with 5,6- or 6,7-disubstituted β-tetralones, provided the 1-(2′-nitrobenzylidene)-2-tetralones, **12a-f and j**. Reduction with zinc in acetic acid gave the desired 5,6-dihydrobenz[a]acridine derivatives, **13a-f and j**. Heating in decalin at 190°C in the presence of Pd/C resulted in conversion of these dihydro compounds to their benz[a]acridine derivatives, **14a-d and j**. Treatment of either **13a** or **14a** with BBr_3 in methylene chloride provided the tetrahydroxy analogs, **13g** and **14g**, respectively. Reaction of **13a** or **14a** with dimethyl sulfate resulted in the formation of their 7-methyl derivatives, **15a** and **16a**.

The starting materials, 2-nitro-4,5-dimethoxybenzaldehyde and 2-nitro-4,5-methylenedioxybenzaldehyde are commercially available. The preparation of 6-chloro-β-tetralone can be performed as described by Rosowsky, et al., *J. Org. Chem.* **1968**, 33, 4288-4290.

7-Nitro-β-tetralone was prepared from 7-nitro-α-tetralone, using a similar procedure to that reported by Nichols et al. (*Organic Preparations and Procedures* **1977**, 277-280) as illustrated in Figure 3. 7-Nitro-β-tetralone served as an intermediate in the preparation of **20**, **21**, **22**, and **23**, as illustrated in Figure 3.

5,6-Methylenedioxy-2-tetralone (**11e**) was used as the requisite intermediate for the preparation of 5,6-dihydro-9,10-dimethoxy-3,4-methylendioxybenz[a]acridine, **13e**. This tetralone was prepared in six steps as illustrated in Figure 4. 2,3-Methylenedioxybenzaldehyde was condensed with malonic acid to give 2,3-methylenedioxycinnamic acid (J. Koo et al. *Org*

Synthesis Coll Vol. IV, 1963, 327-329). 2,3-Methylenedioxycinnamic acid was hydrogenated using 10% Pd/C to give the dihydrocinnamic acid derivative, which was then transformed into its ethyl ester, **17** (M.A. Brook; T.N. Chan, *Synthesis Comm.*, 1983, 201-203). Ethyl-2,3-methylenedioxydihydrocinnamate was then converted to its β-ketosulfoxide, **18** (J.G. Cannon et al. *J. Med. Chem.*, 1977, *20*, 1111-1116). The β-ketosulfoxide derivative, **18**, when subjected to Pummerer rearrangement by treatment with trifluoroacetic acid yielded 1,2,3,4-tetrahydro-1-methylthio-5,6-methylenedioxy-2(1H)-napthalenone, **19** (Y. Oikawk, *Tetrahedron*, 1974, *30*, 2653-2660). Hydrogenolysis of **19** using 10% Pd/C in glacial acetic acid gave **11e** (D.E. Nicholes, *J. Med. Chem.*, 1990, *33*, 703-710.

Pharmaceutically acceptable salts of compounds of formula I may be used as well in practicing the claimed methods. Pharmaceutically acceptable salts may be formed using organic or inorganic bases, such as NaOH, $Na(CO_3)_2$, $NaHCO_3$, KOH and the like; as well as acids such as hydrochloric and sulfoacetic acids and the like. Although the compounds described herein and/or their salts may be administered as the pure chemicals, it is preferable to present the active ingredient as a pharmaceutical composition. The invention thus further provides the use of a pharmaceutical composition comprising one or more compounds and/or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically acceptable carriers and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

Pharmaceutical compositions include those suitable for oral or parenteral (including intramuscular, subcutaneous and intravenous) administration. The compositions may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with liquid carriers, solid matrices, semi-solid carriers, finely

divided solid carriers or combination thereof, and then, if necessary, shaping the product into the desired delivery system.

Pharmaceutical compositions suitable for oral administration may be presented as discrete unit dosage forms such as hard or soft gelatin capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or as granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art., e.g., with enteric coatings.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspension, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservative.

The compounds may also be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small bolus infusion containers or in multi-does containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

For topical administration to the epidermis, the compounds may be formulated as ointments, creams or lotions, or as the active ingredient of a transdermal patch. Suitable transdermal delivery systems are disclosed, for example, in Fisher et al. (U.S. Patent No. 4,788,603) or Bawas et al. (U.S. Patent

No. 4,931,279, 4,668,504 and 4,713,224). Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The active ingredient can also be delivered via iontophoresis, e.g., as disclosed in U.S. Patent Nos. 4,140,122, 4,383,529, or 4,051,842.

Compositions suitable for topical administration in the mouth include unit dosage forms such as lozenges comprising active ingredient in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; mucoadherent gels, and mouthwashes comprising the active ingredient in a suitable liquid carrier.

When desired, the above-described compositions can be adapted to provide sustained release of the active ingredient employed, e.g., by combination thereof with certain hydrophilic polymer matrices, e.g., comprising natural gels, synthetic polymer gels or mixtures thereof.

The pharmaceutical compositions according to the invention may also contain other adjuvants such as flavorings, coloring, antimicrobial agents, or preservatives.

It will be further appreciated that the amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

In general, however, a suitable dose will be in the range of from about 0.5 to about 100 mg/kg, e.g., from about 10 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day.

The compound is conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

Ideally, the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.5 to about 75 μM, preferably, about 1 to 50 μM, most preferably, about 2 to about 30 μM. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1-100 mg of the active ingredient. Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the active ingredient(s).

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

The following examples are intended to illustrate but not limit the invention.

EXAMPLES

Example I - *General.*

Melting points were determined with a Thomas-Hoover unimelt capillary melting point apparatus. Infrared spectral data (IR) were obtained on a Perkin-Elmer 1600 Fourier transform spectrophotometer and are reported in cm^{-1}. Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^1H and 50 MHz ^{13}C) were recorded in $CDCl_3$ (unless otherwise noted) with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in hertz. Mass spectra were obtained from Washington University Resource for Biomedical and Bio-Organic Mass

Spectrometry. Column chromatography refers to flash chromatography conducted on SiliTech 32-63 μm, (ICN Biomedicals, Eschwegge, Ger.) using the solvent systems indicated. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and were within ± 0.4%.

2,3,9,10-Tetramethoxy-7-methyl-5,6-dihydrobenz[c]acridine (6). 2-Amino-4,5-dimethoxyacetophenone (1.0 gm, 5.1 mmol) was dissolved in 10 mL CH_2Cl_2 and hydrogen chloride (1.0 M solution, anhydrous in ether) were added with vigorous stirring at room temperature. The hydrochloride salt of the aminoacetophenone precipitated out. The solvent was removed *in vacuo* and the solid residue obtained dried for an hour under vacuum. The dry hydrochloride salt was then triturated with 6,7-dimethoxy-1-tetralone (1.59 gm, 7.68 mmol) and the mixture was then transferred into a sealed tube and heated at 140 °C for 1 hour. The resulting fused plug was then dissolved in boiling methanol (300 mL). This solution was then concentrated to 200 mL and left overnight providing needle-shaped crystals. These crystals were filtered and washed with three 5 mL portions of acetone, and dried to give golden yellow needles of the benz[c]acridine hydrochloride derivative in 99% yield. The hydrochloride salt was dissolved in 200 mL boiling methanol. After the solution had cooled to room temperature concentrated NH_4OH was added dropwise until pH 10 was obtained. Light yellow crystals were began to form. The suspension was then diluted with 200 mL water and extracted with thrice with 100 mL portions of CH_2Cl_2. The combined extracts were washed once with 50 mL brine, dried using anhydrous Na_2SO_4, filtered, and the solvent removed *in vacuo* to give the free base; mp 240 °C; IR (Nujol): 2922, 1620; ^{1}H NMR: δ 2.52 (3H, s), 2.86 (2H s), 3.02 (2H, t), 3.90 (3H, s), 3.98 (3H, s), 4.03 (3H, s), 4.05 (3H, s), 6.70 (1H, s), 7.08 (1H, s), 7.43 (1H, s), 8.04 (1H, s); ^{13}C NMR: δ 16.5, 25.4, 28.0, 56.6, 56.7, 57.8, 58.3, 102.3, 102.7, 110.9, 111.8, 119.5, 123.2, 127.7, 134.7, 135.6, 146.3, 148.5, 149.7, 151.3, 153.5, 155.2; HRMS calcd for $C_{22}H_{23}NO_4$: 365.1630; found: 365.1628.

5,6-Methylenedioxy-2-tetralone (11e): 0.669 g (2.83 mM) of **19** was taken up in 10 mL glacial acetic acid in a hydrogenation flask. 0.46 g of 10%

Pd-C was added and this mixture was shaken in a Parr apparatus at 40 psig of hydrogen for 40 hours. The reaction mixture was filtered through a celite bed, which was washed thrice with 5 mL portions of glacial acetic acid. The glacial acetic acid was rotaevaporated to give the crude tetralone, **11e**. The crude tetralone was then treated with sodium bisulfite to convert it to the more stable bisulfite adduct. Pure tetralone was generated as required from its bisulfite adduct by treatment with 10% sodium carbonate solution followed by extraction with dichloromethane; mp = 90-91°C (lit[36] 88-91°C), IR (Nujol) 1715; ^{1}H NMR: δ 2.50 (2H, t), 2.98 (2H, t), 3.50 (2H, s), 5.93 (2H, s), 6.56 (1H, d, J = 6), 6.65 (1H, d, J = 6); ^{13}C NMR: δ 21.6, 37.9, 45.0, 101.5, 107.4, 118.5, 121.1, 127.8, 144.1, 146.2, 211.2; Anal. ($C_{11}H_{10}O_3$) C, H.

Example II - *General procedure for the synthesis of 1-(2'-nitrobenzylidene)-2-tetralone derivatives (Figure 2).*

A glacial acetic acid (10 mL) solution of 2.45 mmol of the respective 2-tetralone, 2-nitrobenaldehyde, and sodium acetate was refluxed for 3-8 h under nitrogen atmosphere. The reaction mixture was then allowed to cool to room temperature. The mixture was the carefully loaded on a silica gel (75 gm) column and chromatographed using a 1:1 mixture of ethyl ether and hexanes. The yellow colored compound generally eluting fourth from the column was collected to give the respective tetralone derivatives in 20-25% yield.

1-(2'-Nitro-4',5'-dimethoxybenzylidene)-6,7-dimethoxy-2-tetralone (12a). Prepared from 6,7-Dimethoxy-2-tetralone and 6-nitroveratraldehyde; mp 65-66 °C; IR (Nujol): 2855,1720, 1540; ^{1}H NMR: δ 2.71 (2H, t), 2.98 (2H t), 3.35 (3H, s), 3.66 (3H, s), 3.89 (3H, s), 3.97 (3H,s), 6.41 (1H, s), 6.62 (1H, s), 6.73 (1H, s), 7.74 (1H, s), 7.91 (1H, s); ^{13}C NMR: δ 28.4, 39.7, 56.5, 56.6, 56.8, 56.9, 107.7, 108.6, 111.2, 113.1, 128.1, 129.3, 130.3, 132.2, 133.3, 148.9, 149.0, 150.0, 153.3, 200.7.

1-(2'-Nitro-4',5'-methylenedioxybenzylidene)-6,7-dimethoxy-2-tetralone (12b). Prepared from 6,7-dimethoxy-2-tetralone and 6-nitropiperonal; mp 76-77 °C; IR (Nujol): 2875,1723, 1553; ^{1}H NMR: δ 2.68 (2H, t), 3.00 (2H

t), 3.41 (3H, s), 3.90 (3H, s), 6.08 (2H, s), 6.37 (1H, s), 6.51 (1H, s), 6.73 (1H, s), 7.68 (1H, s), 7.83 (1H, s); ^{13}C NMR: δ 28.0, 38.0, 56.1, 56.4, 103.7, 105.9, 110.1, 111.3, 112.6, 124.3, 130.8, 131.1, 132.2, 133.8, 147.6, 148.4, 149.7, 152.5.

1-(2'-Nitro-4',5'-dimethoxybenzylidene)-2-tetralone (12c). Prepared from 2-tetralone and 2-nitro-4,5-dimethoxybenzaldehyde; mp 58-60 °C; IR (Nujol): 1724, 1540; ^{1}H NMR: δ 2.71 (2H, t), 3.08 (2H t), 3.56 (3H, s), 4.02 (3H, s), 6.51 (1H, s), 6.92-6.96 (2H, m), 7.17-7.24 (2H, m), 7.73 (1H, s), 8.00 (1H, s); ^{13}C NMR: δ 28.3, 27.8, 56.7, 56.9, 108.3, 112.5, 126.7, 127.6, 128.5, 128.8, 129.9, 132.3, 133.4, 134.5, 138.8, 141.6, 149.4, 153.5.

1-(2'-Nitro-benzylidene)-6,7-dimethoxy-2-tetralone (12d). Prepared from 6,7-dimethoxy-2-tetralone and 2-nitrobenzaldehyde; mp 63-64 °C; IR (Nujol): 1712, 1540; ^{1}H NMR: δ 2.69 (2H, t), 3.01 (2H t), 3.25 (3H, s), 3.88 (3H, s), 6.26 (1H, s), 6.72 (1H, s), 7.25-7.26 (1H, m), 7.44-7.50 (2H, m), 7.87 (1H, s), 8.12-8.17 (1H, m); ^{13}C NMR: δ 28.0, 37.9, 55.7, 56.3, 108.6, 111.3, 112.5, 124.2, 124.9, 125.4, 129.3, 130.1, 131.8, 133.5, 133.9, 134.7, 147.5, 149.6, 200.7.

1-(2'-Nitro-4',5'-dimethoxybenzylidene)-5,6-methylenedioxy-2-tetralone (12e). Prepared from 5,6-methylendioxy-2-tetralone and 2-nitro-4,5-dimethoxybenzaldehyde; mp 66-68°C: IR (Nujol): 1710, 1553; ^{1}H NMR: δ 2.98 (2H, t), 3.19 (2H, t), 4.02 (3H, s), 4.03 (3H, s), 6.03 (2H, s), 6.82 (1H, d, J = 8.1), 7.07 (1H, s), 7.29 (1H, d, J = 8.1), 7.49 (1H, s), 8.20 (1H, s); ^{13}C NMR: δ 21.8, 30.8, 56.5, 56.7, 101.8, 105.7, 107.6, 117.9, 119.1, 123.9, 125.9, 126.9, 128.7, 143.4, 145.4, 147.7, 150.1, 152.9, 156.1, 176.4; Anal. ($C_{20}H_{17}NO_7$) C, H, N.

1-(2'Nitro-4',5'-dimethoxybenzylidene)-5,6-dimethoxy-2-tetralone (12f). Prepared from 5,6-dimethoxy-2-tetralone and 2-nitro-4,5-dimethoxybenzaldehyde; mp 70-72°C; IR (Nujol): 1715, 1550; ^{1}H NMR: δ 2.59 (2H, t), 3.06 (2H, t), 3.78 (3H, s), 3.86 (3H, s), 3.88 (3H, s), 3.92 (3H, s), 6.47 (1H, s), 6.77 (1H, d, J = 8.2), 7.41 (1H, d, J = 8.2), 7.67 (1H, s); ^{13}C NMR: δ 19.7, 32.0, 56.3, 56.7, 56.8, 56.85, 108.1, 121.9, 125.6, 126.1, 128.0, 129.2,

132.1, 132.9, 133.7, 140.3, 141.4, 149.3, 152.9, 153.5, 200.9; Anal. ($C_{21}H_{21}NO_7$) C, H, N.

1-(2'-Nitro-4',5'-dimethoxybenzylidene)-6-chloro-2-tetralone (12j). Prepared from 6-chloro-2-tetralone and 2-nitro-4,5-dimethoxybenzaldehyde; mp 56-58 °C; IR (Nujol): 2922, 1730, 1100; ^{1}H NMR: δ 2.68 (2H, t), 3.05 (2H t), 3.62 (3H, s), 3.98 (3H, s), 6.48 (1H, s), 6.82 (1H, d, J = 8.4), 6.90-6.96 (1H, dd, J = 8.4, 2.1), 7.26 (1H, d, J = 2.1), 7.74 (1H, s), 8.01 (1H, s); ^{13}C NMR: δ 28.2, 37.4, 56.8, 56.9, 108.3, 112.2, 127.0, 127.2, 128.6, 130.9, 131.1, 133.3, 134.2, 134.5, 140.5, 141.5, 149.6, 153.7.

1-(2'-Nitro-4',5'-dimethoxybenzylidene)-7-nitro-2-tetralone (Figure 3). Prepared from 7-nitro-2-tetralone and 2-nitro-4,5-dimethoxybenzaldehyde; mp 125 °C; IR (Nujol): 1724, 1540, 1545; ^{1}H NMR: δ 2.69 (2H, t), 3.16 (2H t), 3.59 (3H, s), 4.01 (3H, s), 6.45 (1H, s), 7.41 (1H, d, J = 8.1), 7.68 (1H, d, J = 2.2), 7.72 (1H, s), 7.94-7.99 (1H, dd, J = 8.4, 2.2), 8.08 (1H, s); ^{13}C NMR: δ 28.4, 36.9, 56.9, 57.0, 108.7, 111.6, 123.2, 124.2, 126.1, 129.7, 132.5, 133.9, 135.8, 141.4, 145.8, 146.8, 150.1, 154.1, 198.4.

Example III - *General procedure for the synthesis of 5,6-dihydrobenz[a]acridine derivatives (Figure 2).*

The respective 1-(2'-nitrobenzylidene)-2-tetralone derivative (0.3 mmol) was dissolved in 10 mL glacial acetic acid and refluxed with zinc dust (1.64 mmol) under a nitrogen atmosphere for 1-4 h. The reaction mixture was allowed to cool to room temperature and then the entire mixture was loaded carefully on silica gel (100 gm) column and chromatographed first with 500 mL of ethyl ether to remove acetic acid followed by elution with hexanes/ethyl acetate. The polarity of the mobile phase was reduced, if necessary, by mixing suitable proportions of hexanes. The relevant fractions were pooled and concentrated *in vacuo* to yield 83-95% of corresponding 5,6-dihydrobenz[a]acridines.

2,3,9,10-Tetramethoxy-5,6-dihydrobenz[a]acridine (13a). Prepared from 1-(2'-Nitro-4',5'-dimethoxybenzylidene)-6,7-dimethoxy-2-tetralone; mp 182-183 °C; IR (Nujol): 3210,1615; ^{1}H NMR: δ 2.99 (2H, t), 3.23 (2H t), 3.94

(3H, s), 4.00 (3H, s), 4.02 (3H, s), 4.04 (3H,s), 6.79 (1H, s), 7.10 (1H, s), 7.31(1H, s), 7.54 (1H, s), 8.17 (1H, s); ^{13}C NMR: δ 28.8, 32.3, 56.5, 56.7, 105.7, 107.2, 107.5, 111.8, 123.9, 125.8, 127.1, 128.0, 130.5, 143.1, 148.8, 149.6, 152.8, 156.7, 176.7; HRMS calcd for $C_{21}H_{21}NO_4$: 351.1471; found: 351.1475.

2,3,-Dimethoxy-9,10-methylenedioxy-5,6-dihydrobenz[a]acridine (13b). Prepared from 1-(2'-Nitro-4',5'-methylenedioxybenzylidene)-6,7-dimethoxy-2-tetralone; mp 218-219 °C; IR (Nujol): 2780,1630; ^{1}H NMR: δ 2.97 (2H, t), 3.17 (2H t), 3.93 (3H, s), 4.00 (3H, s), 6.07 (2H, s), 6.79 (1H, s), 7.07 (1H, s), 7.29 (1H, s), 7.32 (1H, s), 8.07 (1H, s); ^{13}C NMR: δ 29.0, 33.1, 56.5, 56.7, 102.1, 103.3, 105.7, 107.6, 111.8, 125.2, 125.9, 127.0, 128.0, 130.6, 145.0, 147.9, 148.8, 149.6, 150.8, 156.9; HRMS calcd for $C_{20}H_{17}NO_4$: 335.1158; found: 335.1162.

9,10-Dimethoxy-5,6-dihydrobenz[a]acridine (13c). Prepared from 1-(2'-Nitro-4',5'-dimethoxybenzylidene)-2-tetralone; mp 95-96 °C; IR (Nujol): 1633, 1516; ^{1}H NMR: δ 3.06 (2H, t), 3.24 (2H t), 4.02 (3H, s), 4.03 (3H, s), 7.10 (1H, s), 7.28-7.37 (3H, m), 7.50 (1H, s), 7.82 (1H, d, J = 7.0), 8.29 (1H, s); ^{13}C NMR: δ 29.2, 32.3, 56.5, 56.7, 105.9, 107.4, 124.0, 124.3, 127.3, 127.7, 128.5, 128.9, 129.1, 133.8, 137.6, 144.2, 150.5, 153.1; HRMS calcd for $C_{19}H_{17}NO_2$: 291.1259; found: 291.1250.

2,3-Dimethoxy-5,6-dihydrobenz[a]acridine (13d). Prepared from 1-(2'-Nitro-benzylidene)-6,7-dimethoxy-2-tetralone; mp 55-56 °C; IR (Nujol): 2815, 1615; ^{1}H NMR: δ 2.96 (2H, t), 3.23 (2H t), 3.90 (3H, s), 3.98 (3H, s), 6.75 (1H, s), 7.30 (1H, s), 7.44 (1H, t), 7.60 (1H, t), 7.80 (1H, d, J = 10.2), 8.0 (1H, d, J = 10.2), 8.19 (1H, s); ^{13}C NMR: δ 27.3, 28.9, 56.5, 56.6, 106.2, 110.7, 125.2, 125.5, 127.6, 127.8, 131.2, 132.4, 133.7, 133.9, 134.2, 136.3, 151.3, 153.6, 157.8; HRMS calcd for $C_{19}H_{17}NO_2$: 291.1259; found: 291.1246.

5,6-Dihydro-9,10-dimethoxy-3,4-methylenedioxybenz[a]acridine (13e). Prepared from 1-(2′-nitro-4′,5′-dimethoxybenzylidine)-5,6-methylenedioxy-2-tetralone; mp 220-222°C; IR (Nujol): 1715, 1532; ^{1}H NMR: δ 3.02 (2H, t), 3.19 (2H, t), 4.02 (3H, s), 4.03 (3H, s), 6.03 (2H, s), 6.83 (1H, d, J = 8.1), 7.07 (1H, s), 7.34-7.38 (2H, m), 8.19 (1H, s); ^{13}C NMR: δ 30.2, 32.3, 56.5,

56.6, 101.8, 105.8, 107.5, 107.8, 117.9, 119.2, 123.8, 126.9, 128.3, 128.7, 143.2, 145.4, 147.6, 149.9, 152.7, 156.3; Anal. ($C_{20}H_{17}NO_4$) C, H, N.

5,6-Dihydro-3,4,9,10-tetramethoxybenz[a]acridine (13f). Prepared from 1-(2′-nitro-4′,5′-dimethoxybenzylidine)-5,6-dimethoxy-2-tetralone; mp 195-196°C; IR (Nujol): 1730, 1515; ^{1}H NMR: δ 2.82 (2H, t), 3.20 (2H, t), 3.86 (3H, s), 3.91 (3H, s), 4.03 (3H, s), 4.04 (3H, s), 6.93 (1H, d, J = 8.6), 7.10 (1H, s), 7.52 (1H, s), 7.56 (1H, d, J = 8.6), 8.27 (1H, s); ^{13}C NMR: δ 21.8, 31.6, 56.4, 56.6, 56.8, 61.1, 105.8, 106.4, 106.5, 107.6, 111.4, 111.9, 120.4, 124.1, 127.1, 129.3, 131.6, 150.3, 150.4, 153.4, 156.3. Anal. ($C_{21}H_{21}NO_4$) C, H, N.

2-Amino-9,10-dimethoxy-5,6-dihydrobenz[a]acridine (Figure 3, 20). Prepared from 1-(2'-Nitro-4',5'-dimethoxybenzylidene)-7-nitro-2-tetralone; mp 185 °C; IR (Nujol): 3345, 2895, 1330; ^{1}H NMR (CD_3OD): δ 2.96 (2H, t), 3.36 (2H t), 3.98 (3H, s), 4.02 (3H, s), 7.24 (1H, d, J = 10.0), 7.32-7.38 (2H, m), 7.88 (1H, s), 8.17 (2H, s); ^{13}C NMR (CD_3OD): δ 24.5, 27.7, 57.0, 57.1, 99.4, 107.9, 117.1, 121.9, 123.2, 125.9, 128.9, 130.1, 133.2; HRMS calcd for $C_{19}H_{18}N_2O_2$: 306.1369; found: 306.1369.

3-Chloro-9,10-dimethoxy-5,6-dihydrobenz[a]acridine (13j). Prepared from 1-(2'-Nitro-4',5'-dimethoxybenzylidene)-6-chloro-2-tetralone; mp 197 °C; IR (Nujol): 2895, 1105; ^{1}H NMR: δ 2.96 (2H, t), 3.12 (2H t), 3.97 (3H, s), 3.98 (3H, s), 7.00 (1H, s), 7.20-7.26 (2H, m), 7.32 (1H, s), 7.64 (1H, d, J = 8.1), 8.11 (1H, s); ^{13}C NMR: δ 29.1, 32.6, 56.5, 56.6, 105.8, 107.8, 123.6, 125.6, 125.8, 127.7, 128.6, 128.8, 132.3, 133.9, 139.3, 144.4, 150.0, 153.0, 156.6; HRMS calcd for $C_{19}H_{16}ClNO_2$: 325.0869; found: 325.0887.

Example IV - *General procedure for the synthesis of benz[a]acridines and benz[c]acridines from their 5,6-dihydro derivatives(Figure 2).*

The respective 5,6-dihydrobenz[a]acridine or 5,6-dihydrobenz[c]acridine derivatives (0.22 mmol) were refluxed in 15 mL decalin with 76 mg of 10% palladium on carbon under nitrogen atmosphere for 2-9 h. The reaction mixture was then quickly filtered under suction while hot through a celite bed using a sintered glass funnel. The filter bed was washed thoroughly thrice using 20 mL

portions of boiling chloroform followed by two 20 mL portions of boiling ethyl acetate. The combined filtrate was then concentrated *in vacuo* and dried under vacuum to give the respective benz[a]acridine derivatives.

2,3,9,10-Tetramethoxy-7-methylbenz[c]acridine (Figure 1, 7). Prepared from **6**; mp >250 °C; IR (Nujol): 3520,1633, 1610; ^{1}H NMR: δ 2.87 (3H, s), 4.03 (3H, s), 4.04 (3H, s), 4.13 (3H,s), 4.23 (3H,s), 7.17 (1H, s), 7.20 (1H, s), 7.52(1H, d, J = 9.2), 7.59 (1H, s), 7.80 (1H, d, J = 9.2), 8.87 (1H, s); ^{13}C NMR: δ 14.4, 56.4, 56.7, 101.7, 106.0, 108.2, 108.3, 120.8, 122.1, 122.4, 125.5, 125.8, 126.9, 128.6, 135.7, 139.0, 145.2, 149.8, 150.9, 153.3; HRMS calcd for $C_{22}H_{21}NO_4$: 363.1470; found: 363.1472.

2,3,9,10-Tetramethoxybenz[a]acridine (14a). Prepared from **13a**; mp 247-249 °C; IR (Nujol): 2810,1630; ^{1}H NMR: δ 3.99 (3H, s), 4.01 (3H, s), 4.03 (3H, s), 4.08 (3H,s), 7.08 (1H, s), 7.12 (1H, s), 7.41(1H, s), 7.74 (1H, d, J = 9.3), 7.81 (2H, m), 8.82 (1H, s); ^{13}C NMR: δ 56.3, 56.4, 56.5, 56.6, 103.9, 104.8, 106.8, 109.2, 122.8, 123.0, 124.5, 126.2, 126.4, 127.7, 130.7, 145.7, 147.2, 149.8, 149.9, 150.3, 153.9; HRMS calcd for $C_{21}H_{19}NO_4$: 349.1314; found: 349.1314.

2,3,-Dimethoxy-9,10-methylenedioxybenz[a]acridine (14b). Prepared from **13b**; mp 245-246 °C; IR (Nujol): 2790,1630; ^{1}H NMR: δ 4.06 (3H, s), 4.16 (3H, s), 6.15 (2H, s), 7.25 (2H, s), 7.48 (1H, s), 7.84 (1H, d, J = 8.2), 7.99 (1H, s), 8.99 (1H, s); ^{13}C NMR: δ 56.5, 56.6, 102.3, 102.4, 104.1, 104.9, 109.4, 112.8, 122.9, 124.4, 126.5, 126.7, 128.5, 130.9, 135.7, 147.0, 147.4, 148.4, 150.1, 152.0; HRMS calcd for $C_{20}H_{15}NO_4$: 333.1002; found: 333.1004.

9,10-Dimethoxybenz[a]acridine (14c). Prepared from **13c**; mp 181-182 °C; IR (Nujol): 2883, 1621; ^{1}H NMR: δ 4.09 (6H, s), 7.26 (1H, s), 7.54 (1H, s), 7.60-7.71 (2H, m), 7.89-7.96 (3H, m), 8.69 (1H, d, J = 8.1), 9.23 (1H, s); ^{13}C NMR: δ 56.6, 56.7, 105.1, 107.1, 122.9, 123.3, 123.4, 125.5, 127.6, 128.5, 128.7, 129.3, 130.4, 131.5, 131.6, 135.7, 146.4, 148.0, 150.6, 154.3; HRMS calcd for $C_{19}H_{17}NO_2$: 289.1104; found: 289.1104.

2,3-Dimethoxy-benz[a]acridine (14d). Prepared from **13d**; mp 190-192 °C; IR (Nujol): 2881, 1632; ^{1}H NMR: δ 4.02 (3H, s), 4.13 (3H, s), 7.18 (1H, s),

7.51-7.59 (1H, m), 7.72-7.81 (1H, m), 7.83 (1H, s), 7.89 (1H, s), 7.93-8.22 (2H, m), 8.23 (1H, d, J = 8.5), 9.13 (1H, s); ^{13}C NMR: δ 56.5, 56.6, 104.4, 109.5, 124.2, 124.5, 126.3, 126.4, 126.8, 126.9, 128.5, 129.4, 130.0, 130.1, 130.2, 132.3, 148.1, 149.2, 150.1, 150.2; HRMS calcd for $C_{19}H_{15}NO_2$: 289.1104; found: 289.1099.

3-Chloro-9,10-dimethoxybenz[a]acridine (14j). Prepared from **13j**; mp 241-243 °C; IR (Nujol): 2893, 1108; ^{1}H NMR: δ 4.04 (3H, s), 4.06 (3H, s), 7.09 (1H, s), 7.43 (1H, s), 7.51-7.57 (1H, dd, J = 8.8, 2.2), 7.68 (1H, d, J = 9.1), 7.75 (1H, d, J = 2.2), 7.90 (1H, d, J = 9.1), 8.42 (1H, d, J = 8.8), 8.92 (1H, s); ^{13}C NMR: δ 56.6, 56.7, 104.9, 106.9, 122.7, 123.3, 124.3, 127.8, 128.2, 128.3, 128.6, 129.7, 130.2, 132.5, 133.2, 146.4, 147.4, 150.7, 154.4; HRMS calcd for $C_{19}H_{14}ClNO_2$: 323.0713; found: 323.0713.

Example V - *General procedure for N-methylation of benz[a]acridines and benz[c]acridines.*

Dimethyl sulfate (4 mL) was added to 0.27 mmol of the respective benz[a]acridine or benz[c]acridine and the mixture heated under nitrogen atmosphere in an oil bath at 150 °C for 20 min-5 hours. Anhydrous ethyl ether (10 mL) was added to the reaction mixture with vigorous stirring after it had cooled to room temperature. The precipitated quaternary salt was filter under suction and washed thrice with 10 mL portions of anhydrous ethyl ether and dried. The quaternary salts were crystallized from boiling methanol in 90% yield.

2,3,9,10-Tetramethoxy-7,12-dimethyl-5,6-dihydrobenz[c]acridinium methosulfate (Figure 1, 8). Prepared from **6**; mp > 250 °C; IR (Nujol): 3510,1645, 1613; ^{1}H NMR (CD_3OD): δ 2.90 (3H, s). 2.96 (2H, t), 3.08 (2H, t), 3.93 (3H,s), 4.00 (3H,s), 4.10 (3H, s), 4.18 (3H,s), 4.62 (3H, s), 7.17 (1H, s), 7.42 (1H, s), 7.61(1H, s), 7.63 (1H, s); ^{13}C NMR: δ 16.6, 27.2, 29.0, 46.2, 57.0, 57.2, 57.3, 57.4, 100.7, 105.5, 112.5, 115.2, 121.2, 125.1, 133.4, 138.5, 139.7, 149.7, 150.5, 152.7, 152.9, 154.8, 157.4; HRMS calcd for $C_{23}H_{26}NO_4$+: 380.1858; found: 380.1856.

2,3,9,10-Tetramethoxy-7,12-dimethylbenz[c]acridinium methosulfate (Figure 1, 9). Prepared from **7**; mp > 240 °C; IR (Nujol): 3495,1640, 1615; ^{1}H NMR (DMSO-d_6): δ 2.53 (3H, s), 3.39 (3H,s), 3.97 (3H,s), 4.03 (3H, s), 4.16 (3H,s), 4.17 (3H, s), 7.69 (1H, s), 7.75 (1H, s), 7.91(1H, s), 7.99 (1H, d, J = 9.5), 8.24 (1H, d, J = 9.5); ^{13}C NMR: δ 15.5, 55.4, 56.4, 56.5, 56.6, 56.7, 102.1, 106.3, 110.3, 111.2, 125.8, 123.2, 122.5, 128.8, 129.0, 130.9, 133.3, 139.0, 141.1, 147.6, 152.1, 153.8, 155.3; HRMS calcd for $C_{23}H_{24}NO_4$+: 378.1698; found: 378.1695.

2,3,9,10-Tetramethoxy-7-methyl-5,6-dihydrobenz[a]acridinium methosulfate (15a). Prepared from **13a**; mp > 250 °C; IR (Nujol): 3490,1620; ^{1}H NMR (DMSO-d_6): δ 3.06 (2H, t), 3.37 (5H, t), 3.85 (3H, s), 3.93 (3H, s), 4.02 (3H, s), 4.05 (3H,s), 7.04 (1H, s), 7.45 (1H, s), 7.62(1H, s), 7.63 (1H, s), 9.29 (1H, s); ^{13}C NMR: δ 25.9, 27.8, 53.1, 56.0, 56.1, 56.7, 56.8, 99.6, 106.6, 106.7, 107.9, 112.2, 112.3, 122.0, 124.4, 127.3, 129.1, 148.8, 150.3, 151.1, 153.0, 155.3; HRMS calcd for $C_{22}H_{24}NO_4$+: 366.1706; found: 366.1706.

2,3,9,10-Tetramethoxy-7-methylbenz[a]acridinium methosulfate (16a). Prepared from **14a**; mp > 250 °C; IR (Nujol): 2820,1620; ^{1}H NMR (DMSO-d_6): δ 3.39 (3H, s), 3.96 (3H, s), 3.97 (3H, s), 4.03 (3H, s), 4.07 (3H, s), 7.59 (1H, s), 7.67 (1H, s), 7.85 (1H, d, J = 9.2), 8.25 (1H, s), 8.31 (1H, d, J = 9.2), 8.75 (1H, s), 10.18 (1H, s); ^{13}C NMR: δ 54.4, 56.8, 56.9, 57.5, 57.9, 105.7, 106.7, 107.9, 110.4, 123.2, 125.3, 125.4, 127.8, 127.9, 128.3, 131.4, 146.8, 149.3, 152.0, 158.5, 159.9; HRMS calcd for $C_{22}H_{22}NO_4$+: 364.1549; found: 364.1542.

Example VI - *General procedure for the synthesis of 2,3,9,10-tetrahydroxy-5,6-dihydrobenz[a]acridine (Figure 2, 13g) and 2,3,9,10-tetrahydroxybenz[a]acridine (Figure 2, 14g).*

The respective benz[a]acridine derivatives (0.195 mmol) were dissolved in 2 mL CH_2Cl_2 and the solution was chilled to -50 °C using a cooling bath of isopropanol and dry-ice. 1.95 mmols of boron tribromide (1.0 M) solution in CH_2Cl_2 was added under a nitrogen atmosphere. The reaction mixture was

stirred at -50 °C for 1 hour. and then slowly allowed to come to room temperature over a period of 4 hours. The reaction mixture was then cooled to -10 °C and was quenched by addition of 5 mL saturated ammonium chloride solution. The resulting solution was evaporated to dryness and the residue obtained was extracted thrice with 20 ml portions of boiling acetone. The resulting yellow suspensions were filtered each time. The undissolved precipitate was dissolved in 5 mL boiling methanol and set aside overnight. Needle shaped crystals of the respective tetrahydroxybenz[a]acridines were formed in 95% yield.

2,3,9,10-tetrahydroxy-5,6-dihydrobenz[a]acridine (13g). Prepared from **13a**; mp > 220 °C; IR (Nujol): 3361,3164, 2719, 1620; ^{1}H NMR (CD_3OD): δ 2.97 (2H, t), 3.35 (2H t), 6.77 (1H, s), 7.45 (3H, m), 8.83(1H, s); ^{13}C NMR (CD_3OD): δ 27.4, 29.2, 102.9, 110.9, 112.5, 116.6, 122.9, 126.5, 128.5, 129.4, 134.8, 135.6, 146.6, 148.6, 151.1, 152.9, 156.0; HRMS calcd for $C_{17}H_{13}NO_4$: 295.0845; found: 295.0842.

2,3,9,10-tetrahydroxybenz[a]acridine (14g). Prepared from **14a**; mp > 270 °C; IR (KBr): 3361,3164, 1620, 1516; ^{1}H NMR (CD_3OD): δ 7.26 (1H, s), 7.37 (1H, s), 7.56(1H, s), 7.61 (1H, d, J=9.2), 8.05 (2H, m), 9.61(1H, s),; ^{13}C NMR (CD_3OD): δ 101.1, 108.7, 110.3, 114.3, 114.7, 122.9, 124.4, 124.8, 126.7, 137.6, 137.8, 138.3, 139.0, 149.4, 150.7, 150.8, 159.3; HRMS calcd for $C_{17}H_{11}NO_4$: 293.0688; found: 293.0685.

Example VII - *Synthesis of 7-nitro-2-tetralone from 7-nitro-1-tetralone (Figure 3).*

7-Nitro-1,2,3,4-tetrahydro-1-napthalenol. To a slurry of 2.88 g (15 mmol) of 7-nitro-1-tetralone in 60 mL absolute ethanol was added 0.58 g (15 mmol) of sodium borohydride. The reaction mixture was then stirred at room temperature for 2 hours. The resulting mixture was then rotaevaporated to dryness and the residue obtained was suspended in 100 mL of water. 3 N hydrochloric acid was added dropwise until the reaction mixture had pH 7. The suspension obtained was then extracted with five 50 mL portions of ethyl ether

and the combined ether layer was washed once with 100 mL water. The ether layer was dried over anhydrous sodium sulfate, filtered and rotaevaporated to give an off-white residue which was recrystallized from a 1:1 mixture of absolute ethanol and water to yield 2.68 g (92%) of the napthalenol; mp 112-113 °C; IR (KBr): 3300; ^{1}H NMR: δ 1.73-2.26 (4H, m), 2.29 (1H, s), 2.78-2.91 (2H, m), 4.83(1H, m), 7.24 (1H, d, *J*=8.1), 8.00-8.04 (1H, dd, *J*= 8.1. 2.4), 8.35(1H, d, *J*= 2.4),; ^{13}C NMR: δ 19.2, 29.9, 32.5, 68.3, 122.7, 124.1, 130.3, 140.9, 145.4.

7-Nitro-3,4-dihydronapthalene. A mixture of 2.89 g(14.9 mmol) of the 1-napthalenol, 3.5 g of amberlyst-15 catio-exchange resin, and 120 mL of benzene was heated at reflux under a nitrogen atmosphere for 2 hours. The reaction mixture was then cooled to room temperature and dried using anhydrous sodium sulfate and filtered. The filtrate was then rotaevaporated to dryness to give the product as an oil in 92% yield. The product was sufficiently pure and was used in the next step without further purification; IR (KBr): 1545; ^{1}H NMR: δ 2.25-2.36 (2H, m), 2.77-2.86 (2H, t), 6.08-6.17 (1H, m), 6.40-6.45 (1H, m), 7.14 (1H, d, *J*=8.1), 7.72 (1H, d, *J*= 2.3), 7.83-7.89 (1H, dd, *J*= 8.1, 2.3),; ^{13}C NMR: δ 23.0, 27.9, 120.6, 122.1, 126.7, 128.6, 131.9, 135.6, 143.5, 147.3.

1,2-Epoxy-7-nitro-1,2,3,4-tetrahydronapthalene. To a solution of 0.5 g (2.85 mmol) of the 7-nitro-3,4-dihydronapthalene in 9 mL of chloroform was added 0.677 g of *m*-chloroperoxybenzoic acid in one portion. The resulting solution was heated at reflux for 45 minutes. The mixture was then cooled to 0 °C and the precipitated *m*-chlorobenzoic acid was removed by filtration. The chloroform layer was rotaevaporated to give a pale yellow solid which chromatographed over silica gel (50 g) and eluted with dichloromethane. The relevant fractions were combined and rotaevaporated to give 0.487 g (89.5%) of the epoxide; mp 73-74 °C; IR (KBr): 3300; ^{1}H NMR: δ 1.70-1.86 (1H, m), 2.42-2.52 (1H, m), 2.54-2.90 (2H, m), 3.75-3.77 (1H, t), 3.91-3.93 (1H, d, *J* =4.2), 7.22-7.26 (1H, d, *J*= 8.4), 8.05-8.11 (1H, dd, *J*= 8.4, 2.4), 8.24 (1H, d, *J*= 2.4); ^{13}C NMR: δ 21.6, 25.1, 52.3, 55.2, 123.8, 124.8, 129.8, 134.9, 145.0.

7-Nitro-2-tetralone. To a solution of 0.5 g (2.6 mmol) of the above epoxide in 5 mL dry benzene was added 0.37 g (1.1 mmol) of anhydrous zinc iodide. The mixture was stirred at room temperature under nitrogen atmosphere, in the dark. After filtration and removal of solvent under reduced pressure, thc resulting yellow oil was taken up into 3 mL of cold absolute ethanol when the product crystallized. Repetitive crystallization from the concentrated mother liquor gave a total yield of 0.415 g (83%); mp 96-97 °C; IR (KBr): 1710, 1330, 1500; ^{1}H NMR: δ 2.59-2.66 (2H, t), 3.15-3.21 (2H, t), 3.72 (2H, s), 7.38-7.43 (1H, d, $J = 8.3$), 7.98 (1H, d, $J = 2.2$), 8.02-8.03 (1H, dd, $J = 8.3, 2.2$).

Example VIII - *Assays - Materials.*

The plasmid pET11a and the *E. coli* strain BL21(DE3) used for enzyme expression were purchased from Novagen. IPTG was purchased from Sigma. The ECL system used for the Western blotting analysis of bacterial lysates was from Amersham (UK). All the restriction enzymes and Vent polymerase were from New England Biolabs. Mammalian topoisomerase II was isolated from calf thymus glands according to the published procedure (Halligan et al., *J. Biol. Chem. 260*:2475-2482 (1985)). The single copy yeast plasmids YCpGAL1 expressing various topoisomerase I genes in JN2-134 yeast strain were a kind gift of Dr. M-A. Bjornsti (Thomas Jefferson University, Philadelphia, PA). All bacterial and yeast media were from Difco (Detroit, MI), while cell culture media were purchased from Gibco-BRL (Gaithersburg, MD).

Example IX - *Topoisomerase I expression in E. coli.*

To obtain large quantity of human topoisomerase I, the human topoisomerase I cDNA was cloned into the pET-11a vector, in which transcription of the cDNA is under the control of the inducible T7 promoter (Studier et al., Methods in Enzymol., Vol. 185:60-89, San Diego: Academic Press (1990)). Briefly, a 3.4 kb DNA fragment containing the entire coding sequence of human topoisomerase I and approximately 1 kb of untranslated region downstream of the stop codon was isolated from the plasmid

YCpGAL1-hTOP1 (Bjornsti et al., *Cancer Res. 49:*6318-6323 (1989)) by cutting at the BamHI and EcoRI sites. The vector pET-11a was cut with the same restriction enzymes, dephosphorylated and ligated to the insert in the proper reading frame downstream of the vector cloning site. The ligation mixture was used to transform *E. coli*, the correct clone pET1B was isolated and its identity confirmed by restriction mapping. Since the translational start in pET is positioned at an upstream NdeI site, the expressed topoisomerase I has a 15 amino acid fusion at its N-terminus. pET1B was then transformed into *E. coli* BL21(DE3), and, upon induction with 0.4 mM IPTG for 1 hour, the bacterial lysate was analyzed by 10 % SDS-PAGE. Expression was confirmed by Western blotting using rabbit antibodies against human topoisomerase I. Isolation of the expressed protein was done by a simple procedure. Briefly, *E. coli* cells were lysed by repeated sonic bursts. The sonic extract was made in 1 M NaCl and 6% polyethylene glycol (PEG) to remove nucleic acids. The PEG supernatant was chromatographed directly on a hydroxyapatite column. Expressed human DNA topoisomerase I was eluted at the 0.6 M potassium phosphate step. The eluted enzyme was dialyzed against 50% glycerol, 30 mM potassium phosphate (pH 7.0), 1 mM dithiothreitol (DTT) and 0.1 mM EDTA and stored at -20°C. The relaxation activity of the purified enzyme had a specific activity about 2 orders of magnitude lower than the calf thymus topoisomerase I.

Example X - *Expression of camptothecin-resistant (CPT-K5) topoisomerase I in E. coli.*

Two complementary oligonucleotides containing the point mutation CAG (Asp533)->CGG (Gly) responsible for the resistance phenotype in CPT-K5, were synthesized and engineered in the topo I coding sequence using the sequential PCR method (Current Protocols in Molecular Biology, *In*: Ausubel et al. (eds.), Vol. 1, pp. 8.5.7. Boston:Wiley Interscience (1991)). The two oligonucleotides are 5'-CTTCCTCGGGAAG<u>GGC</u>TCCATCAGATAC-3' (primer X1)(SEQ ID NO:1), and

5'-GTATCTGATGGAGCCCTTCCCGAGGAAG-3' (primer X2)(SEQ ID NO:2), where the underlined sequence represents the mutated codon. Each oligonucleotide was used in separate PCR reactions to amplify two DNA segments adjacent to the mutation site, using the oligonucleotides 5'-ACTGTGATCCTAGGG-3' ("A")(SEQ ID NO:3) and 5'-CTTCATCGACAAGCTTGCTCTGAG-3' ("H")(SEQ ID NO:4) as the relative primer pairs for X1 and X2, respectively. "A" and "H" are complementary to the human topo I sequence around the unique restriction sites AvrII and HindIII. After the first round of PCR, the two amplified products X1-H and X2-A were denatured and annealed by their 15 base-pair complementary sequence, due to the overlap of the oligonucleotides X1 and X2. This short stretch of double-stranded DNA segment was then extended by Vent polymerase at 72°C for 2 minutes to the 748 base pairs full length product A-H. The two external primers "A" and "H" were then used to amplify the full length DNA fragment containing the mutated topo I fragment. The amplified mutant topoisomerase I cDNA was then digested with AvrII and HindIII, and cloned into pET1B by replacing the corresponding AvrII/HindIII fragment in the topoisomerase I cDNA sequence. The plasmid pET1B-CPTK5, which contained the mutant CPT-K5 topoisomerase I cDNA instead of the wildtype human topoisomerase I cDNA, was transformed into *E. coli* BL21(DE3) for expression. Upon induction with IPTG, the protein in the lysates was confirmed by Western blotting. The CPT-K5 topoisomerase I was then purified from the bacterial lysate as described for the wildtype enzyme.

Example XI - *Topo I and topo II cleavage assay.*

Cleavage assays for the recombinant topoisomerases I and calf thymus topoisomerases I and II were done as described (Liu et al., *J. Biol. Chem.* *258:*15365-15370 (1983)). The plasmid YEpG DNA used for the cleavage assays was prepared and labeled at its 3'-end using the published procedures.

Example XII - *Yeast cytotoxicity assay.*

It has been established that yeast can survive when topoisomerase I function is obliterated, and that the topoisomerase I poisons only kill cells having a functional topoisomerase I (Bjornsti et al. *Cancer Res. 49:*6318-6323 (1989)). Thus, comparison of the relative extent of growth of each of the test strains in the presence of various drugs with control plates minus drug shows 1) whether the drug has any cytotoxic effects on yeast, 2) whether the cytotoxicity is topo I specific and 3) whether there is any differential specificity of the drug for yeast compared with human topo I.

The topoisomerase I-specific *in vivo* cytotoxicity assay was adapted from Knab et al. (Knab et al., *J. Biol. Chem. 268:*22322-22330 (1993)). In this system, various topo I genes cloned into the single copy yeast plasmid vector, YCpGAL1 (Knab et al., *J. Biol. Chem. 268:*22322-22330 (1993)), are expressed under the control of the GAL1 promoter in the JN2-134 strain of S. cerevisiae (MATa, rad52::LEU2, trp1, ade2-1,his7, ura3-52, ise1, top1-1, leu2) (Bjornsti et al., *Cancer Res. 49:*6318-6323 (1989)). The topo I constructs in the vector are, respectively, the wild-type yeast topo I (YCpGAL-ScTOP1), a non-functional yeast topo I where the active site tyrosine-727 is mutated to a phenylalanine (YCpGAL1-Sctop1Y727F) (Knab et al., *J. Biol. Chem. 268:*22322-22330 (1993)), and the wild type human topoisomerase I (YCpGAL-hTOP1) (Bjornsti et al., *Cancer Res. 49:*6318-6323 (1989)). To qualitatively test the cytotoxicity and the topo I specificity of the drugs, yeast cells containing the specific plasmid were serially diluted (5-fold) and were grown in dropout medium supplemented with uracil and 2% galactose. In addition, the positive and negative control plates contained: A: Control, no drug in the plate; B: Camptothecin (CPT), 0.5 μM; C: Coralyne, 1 μM ; D: Methylenedioxy-dihydro-demethyl-coralyne (MDD-Coralyne), 1 μM, and E: Nitidine, 1 μM. The plates were grown for 3 days at 30°C to assess the lethal effect of the different compounds on the various topoisomerase I enzymes expressed in *S. cerevisiae* and the drug being tested.

Example XIII - *Cytotoxicity assay.*

The IC_{50} of the drugs tested were determined by the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA) (Mosmann, T., *J. Immunol. Methods 65:*55-63 (1983); Denizot et al., *J. Immunol. Methods 89:*271-277 (1986)). Human lymphoblast RPMI 8402 cells and their camptothecin-resistant CPT-K5 cells (Andoh et al., *Proc. Natl. Acad. Sci., USA 84:*5565-5569 (1987)) were kindly provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan). The cell lines A2780 and its camptothecin-resistant derivative CPT-2000 were a generous gift of Dr. Jaulang Hwang (Institute of Molecular Biology, Academia sinica, Taiwan). Cells (2000 cells/well, seeded in 200 ml growth medium) were grown in suspension at 37°C in 5% CO_2 and maintained by regular passage in RPMI medium supplemented with 10% heat inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (0.1 mg/ml). The cells were exposed continuously for 4 days to drug concentrations ranging from 100 ug/ml to 1.0 ng/ml in ten fold dilutions, and assayed at the end of the fourth day. Each concentration and the no drug control were repeated at least twice in 6 replica wells. The results were plotted and the IC_{50} then measured. The drug sensitive human epidermoid carcinoma KB 3-1 cell line and its vinblastine-selected multidrug-resistant variant KB-V1 cells (Akiyama et al., *Genetics 11:*117-126 (1985)) were kindly provided by Dr. Michael Gottesmann (National Cancer Institute). They were grown as monolayer cultures at 37°C in 5% CO_2 and maintained by regular passage in Dulbecco's minimal essential medium supplemented with 10% heat inactivated fetal bovine serum. KB-V1 cells were maintained in the presence of 1 mg/ml vinblastine.

Table 1. Topoisomerase I and Topoisomerase II mediated DNA Cleavage of Coralyne Derivatives and Related Compounds

Compound	Topo I-mediated DNA cleavage[b]	Topo II-mediated DNA cleavage[c]	Cytotoxicity IC_{50}[a] (μM) Cell Lines	
			RPMI	CPT-K5
Coralyne	1	>1000	4.9	20
Nitidine	0.1	5	0.4	3.9
MDD-Coralyne	0.1		8.1	27
6	>1000	>1000	>137	>137
7	>1000	>1000	7.0	5.5
8	>1000	>1000	6.1	12.2
9	>1000	>1000	1.2	1.2
13a	100	>1000	7.1	7.1
13b	100	>1000	9.0	14.9
13c	1000	>1000	6.9	6.9
13d	100	>1000	1.0	8.6
13e	1.0	>1000	3.0	22.4
13f	100	>1000	14.2	14.2
13g	1.0	>1000	20.3	13.6
13j	10	>1000	9.2	15.4
14a	1000	>1000	2.9	2.9
14b	1000	>1000	4.5	7.5
14c	>1000	>1000	10.4	31.1
14d	1000	>1000	10.4	13.8
14g	100	>1000	17.1	>34
14j	1000	>1000	>31	>31
15a	100	>1000	>24	> 24
16a	>1000	>1000	25.3	23.2
20	100	>1000	16.3	>33
CPT	1	>1000	0.004	>10[d]
VM-26	>1000	1	0.3	0.5

a) IC_{50} has been calculated after 4 days of continuous drug exposure. N.D. = Not determined.

b) Topoisomerase I cleavage values are reported as REC, Relative Effective Concentration, i.e., concentrations relative to camptothecin (CPT), whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I.

c) Topoisomerase II cleavage values are reported as REC, Relative Effective Concentration, i.e., concentrations relative to VM-26, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of calf thymus topoisomerase II.

d) No indication of cytotoxicity were considered indicative of IC_{50} values substantially greater than the highest doses assayed

Certain compounds of formula I, in particular, comound **13e**, are potent topoisomerase I poisions. Additionally, compounds of formula I generally possess cytotoxic activity against RPMI 8402 cancer cells and camptothecin resistant CPT-K5 cells. Accordingly, compounds of formula I may be useful as cytotoxic agents, for the treatment of cancers, in particular, the solid mammalian tumors or hematologic malignancies identified herein above.

The fact that benz[a]acridines are non-charged analogs related to coralyne suggests that these agents may have enhanced cell absorption. There is also the potential that they may be less readily cleared than charged compounds *in vivo*. In addition, the absence of the benzisoquinolium moiety, which is present within the structure of coralyne and coralyne analogs, may result in these analogs having less neurotoxicity.

All publications, patents and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Rutgers, The State University of New Jersey

(ii) TITLE OF THE INVENTION: SUBSTITUTED HETEROCYCLES AS ANTI-TUMOR AGENTS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
  (A) ADDRESSEE: Schwegman, Lundberg, Woessner & Kluth, P.A.
  (B) STREET: P.O. Box 2938
  (C) CITY: Minneapolis
  (D) STATE: MN
  (E) COUNTRY: USA
  (F) ZIP: 55402

(v) COMPUTER READABLE FORM:
  (A) MEDIUM TYPE: Diskette
  (B) COMPUTER: IBM Compatible
  (C) OPERATING SYSTEM: DOS
  (D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:
  (A) APPLICATION NUMBER: Unknown
  (B) FILING DATE: 23-SEP-1997
  (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
  (A) APPLICATION NUMBER: 60/026,511
  (B) FILING DATE: 23-SEP-1996

(viii) ATTORNEY/AGENT INFORMATION:
  (A) NAME: Woessner, Warren D
  (B) REGISTRATION NUMBER: 30,440
  (C) REFERENCE/DOCKET NUMBER: 735.036WO1

(ix) TELECOMMUNICATION INFORMATION:
  (A) TELEPHONE: 612-373-6900
  (B) TELEFAX: 612-339-3061
  (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTTCCTCGGG AAGGGCTCCA TCAGATAC 28

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTATCTGATG GAGCCCTTCC CGAGGAAG 28

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACTGTGATCC TAGGG 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

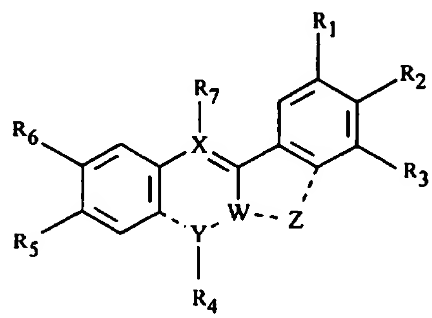
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTTCATCGAC AAGCTTGCTC TGAG 24

CLAIMS

1. A compound of the formula (I):



(I)

wherein

R_1, R_2, R_3, R_5 and R_6 are independently H, OH, NO_2, NH_2, halo, NHCO(C_1-C_8)alkyl or (C_1-C_8)alkoxy; R_1 and R_2 together are -OCH_2O-; R_2 and R_3 together are -OCH_2O-; or R_5 and R_6 together are -OCH_2O-;

R_4 and R_7 are independently H, (C_1-C_8)alkyl or absent;

W is C or N;

X is C or N;

Y is -C=, -N= or a direct bond, provided that where Y is -C=, X is N; and

Z is -CH=CH-, -$(CH_2)_2$- or absent;

or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 wherein W is C.

3. A compound of claim 2 wherein Y is -N= and X is C.

4. A compound of claim 3 wherein Z is -CH=CH- or -$(CH_2)_2$-.

5. A compound of claim 4 wherein R_5 and R_6 are -OCH_3.

6. A compound of claim 5 wherein R_3 is H.

7. A compound of claim 6 wherein R_1 is $-OCH_3$.

8. A compound of claim 7 wherein R_2 is $-OCH_3$.

9. A compound of claim 2 wherein X is N and Y is -C=.

10. A compound of claim 9 wherein Z is -CH=CH- or $-(CH_2)_2-$.

11. A compound of claim 10 wherein R_5 and R_6 are $-OCH_3$.

12. A compound of claim 11 wherein R_3 is H.

13. A compound of claim 12 wherein R_1 is $-OCH_3$.

14. A compound of claim 13 wherein R_2 is $-OCH_3$.

15. A therapeutic method to inhibit cancer cell growth comprising administering to a mammal afflicted with cancer an amount of a compound of claim 1.

16. The method of claim 15 wherein the mammal is a human.

17. The method of claim 15 wherein the cancer is a leukemia or melanoma.

18. The method of claim 15 wherein the cancer is a solid tumor.

19. The method of claim 18 wherein the tumor is a breast, lung, colon, or ovarian tumor.

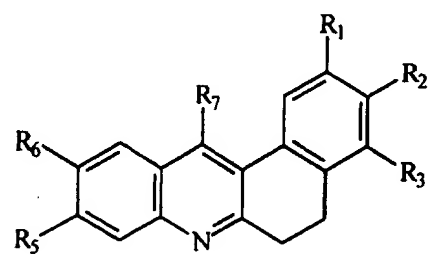
20. The method of claim 15 wherein the compound is administered in combination with a pharmaceutically acceptable carrier.

21. The method of claim 20 wherein the carrier is a liquid vehicle.

22. The method of claim 21 wherein the carrier is adapted for parenteral administration.

23. The method of claim 20 wherein the carrier is a tablet or capsule.

24. A compound of formula II:



(II)

wherein

R_1, R_2, R_3, R_5 and R_6 are independently H, OH, NO_2, NH_2, halo, NHCO(C_1-C_8)alkyl or (C_1-C_8)alkoxy; R_1 and R_2 together are $-OCH_2O-$; R_2 and R_3 together are $-OCH_2O-$; or R_5 and R_6 together are $-OCH_2O-$; and

R_7 is H, or (C_1-C_8)alkyl;

or a pharmaceutically acceptable salt thereof.

25. A compound of claim 24 wherein R_5 and R_6 are OH or (C_1-C_8)alkoxy; or R_5 and R_6 together are $-OCH_2O-$.

26. A compound of claim 24 wherein R_7 is hydrogen, methyl, or ethyl.

27. A compound of claim 1 wherein R_2 and R_3 together are $-OCH_2O-$.

28. The compound 5,6-dihydro-9,10-dimethoxy-3,4-methylenedioxybenz[a]-acridine; or a pharmaceutically acceptable salt thereof.

29. A pharmaceutical composition comprising an effective amount of a compound of claim 1 in combination with a pharmaceutically acceptable carrier.

Figure 1

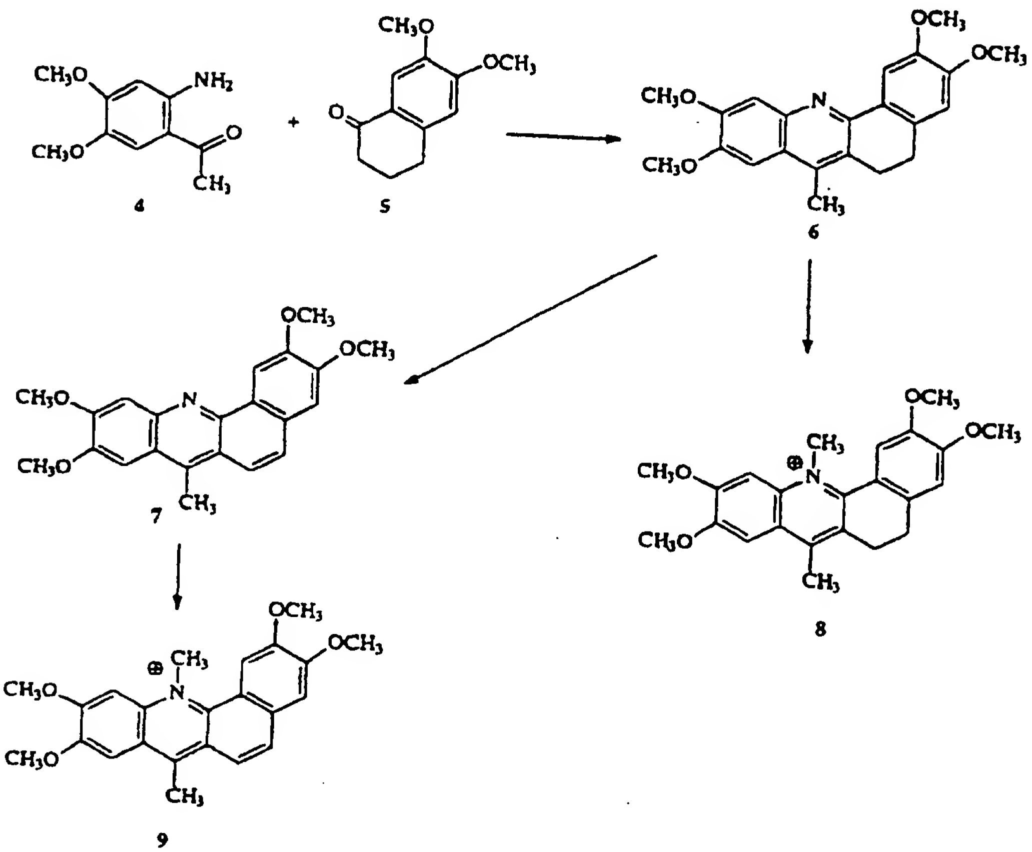
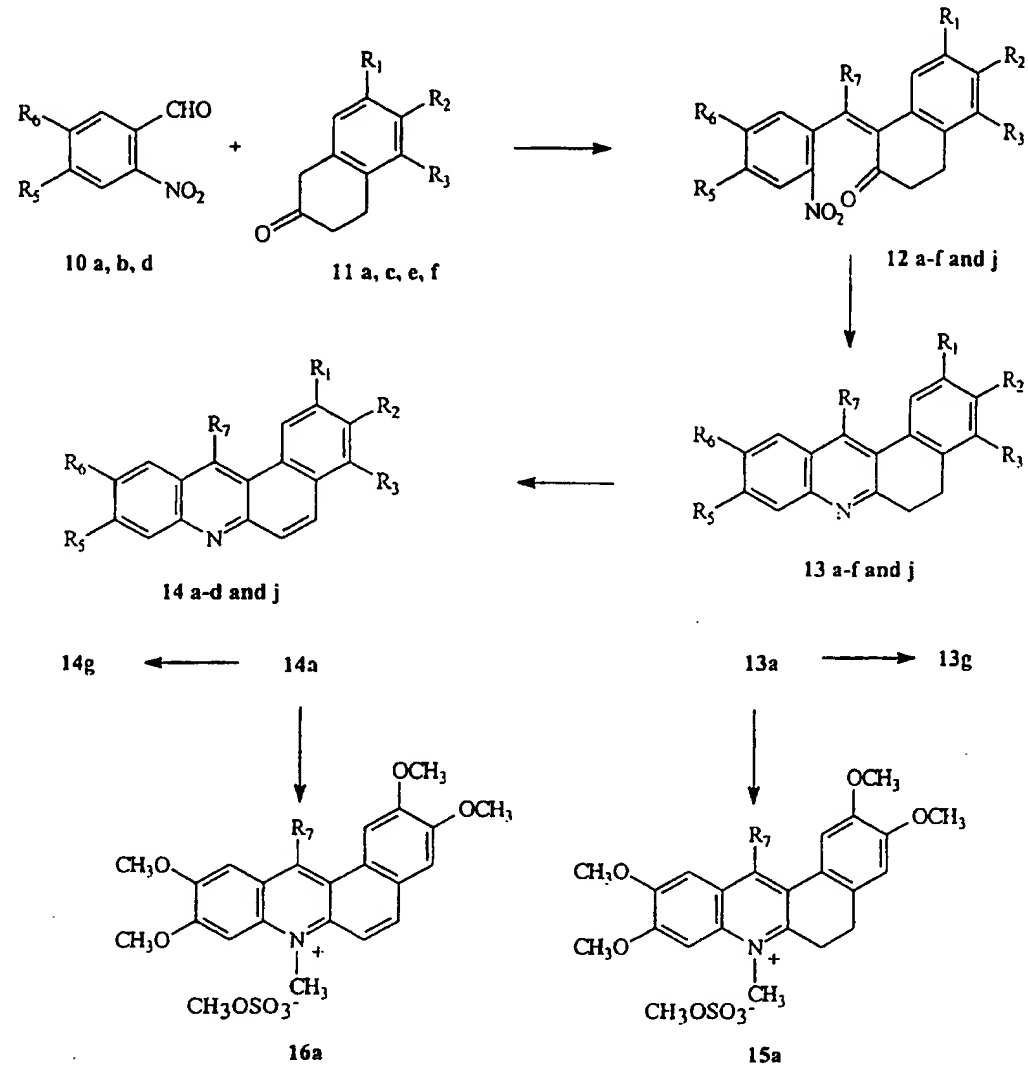

CH3O
NH2
CH3O
O
CH3
4
+
CH3O
OCH3
O
5
OCH3
OCH3
CH3O
N
CH3O
CH3
6
7
8
9

Figure 2



	R_5	R_6	R_1	R_2	R_3
a	OCH_3	OCH_3	OCH_3	OCH_3	H
b	$-OCH_2O-$		OCH_3	OCH_3	H
c	OCH_3	OCH_3	H	H	H
d	H	H	OCH_3	OCH_3	H
e	OCH_3	OCH_3	H	$-OCH_2O-$	
f	OCH_3	OCH_3	H	OCH_3	OCH_3
g	OH	OH	OH	OH	H
j	OCH_3	OCH_3	H	Cl	H

Figure 3

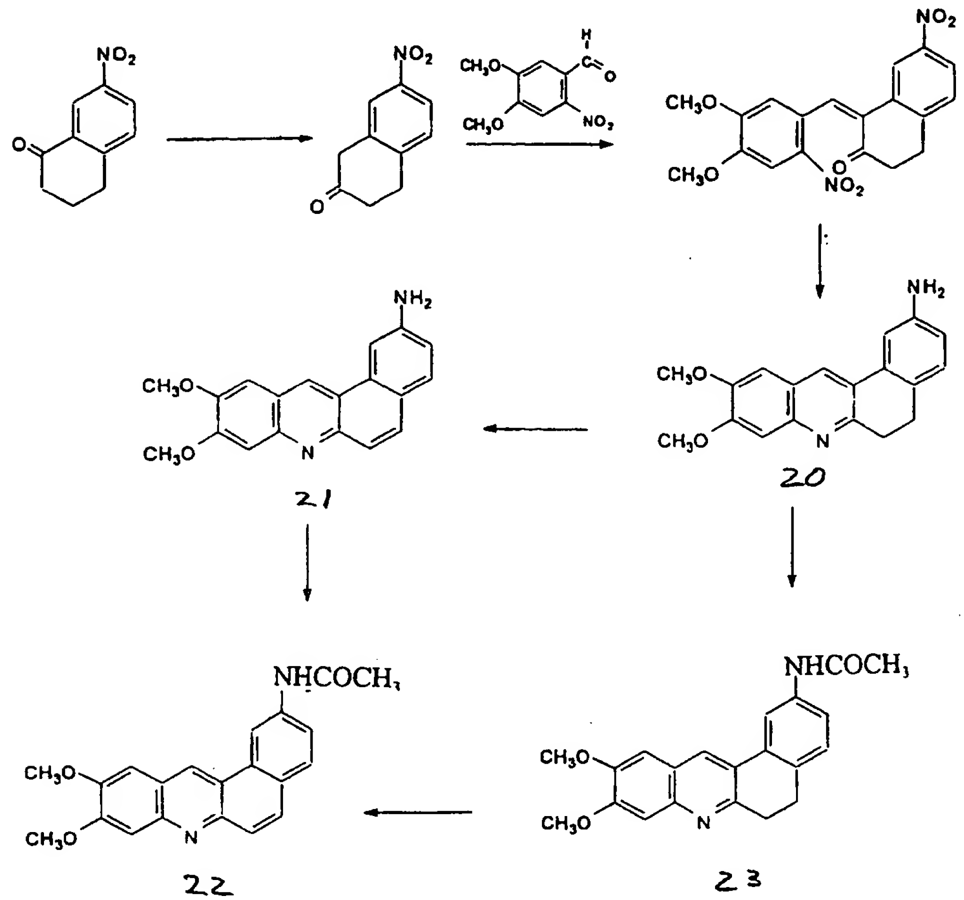
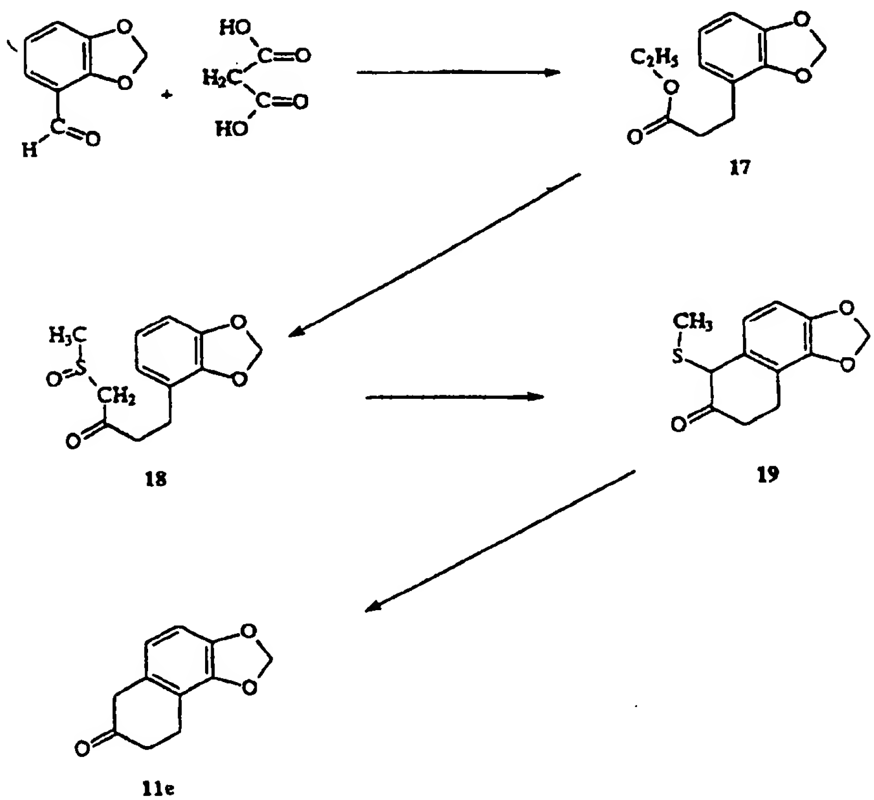


Figure 4



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/17012

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D221/18 C07D491/04 A61K31/435 //(C07D491/04,317:00, 221:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARTINE CROISY-DELCEY ET AL: "Synthesis and carcinogenic activity of oxidised benzacridines:Potential metabolites of the strong carcinogen 7-methylbenz[c]acridine and the inactive isomer 12-methylbenz[a]acridine." JOURNAL OF MEDICINAL CHEMISTRY., vol. 26, no. 2, 1983, WASHINGTON US, pages 303-306, XP002049514 see the whole document ---	1,2,9-14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 December 1997	22.12.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Henry, J

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/17012

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C.BAEZNER ET AL: "Überführung von o-nitro- und o,p-dinitro-benzylchlorid in acridinderivate" BERICHTE DER DEUTSCHEN CHEMISCHEN GESELLSCHAFT., vol. 39, 1906, WEINHEIM DE, pages 2438-2447, XP002049515 see the whole document ---	1-9, 24-26
X	TETSUJI KAMETANI ET AL: "Studies on the synthesis of heterocyclic compounds.DCXXVII.The formation of 2,3,9,10-tetramethoxybenz[c]acridine by treatment of 6,7-dimethoxy-1-(4,5-dimethoxy-2-nitrophenethyl)-2-methylisoquinoline with triethyl phosphite" CHEMICAL AND PHARMACEUTICAL BULLETIN., vol. 23, no. 9, 1975, TOKYO JP, pages 2025-2028, XP002049516 see the whole document ---	1,2,9-14
X	GANDHI K. KAR ET AL: "Regioselective thermal cyclization of 3-substituted arylenaminoimine hydrochlorides,a convenient method for the synthesis of functionalized polycyclic quinoline derivatives" HETEROCYCLES., vol. 41, no. 5, 1995, AMSTERDAM NL, pages 911-920, XP002049517 see page 913 ---	1,2,9-14
X	NG.PH. BUU-HOI: "The chemistry of carcinogenic nitrogen compounds.Part V.Angular hydroxybenzacridines and hydroxydibenzacridines." JOURNAL OF THE CHEMICAL SOCIETY., 1950, LETCHWORTH GB, pages 2096-2099, XP002049518 see the whole document ---	1-4, 24-26
X	NG. PH. BUU-HOI ET AL: "The chemistry of carcinogenic nitrogen compounds. Part X. The Pfitzinger reaction in the synthesis of 1:2-benzacridines." JOURNAL OF THE CHEMICAL SOCIETY., 1952, LETCHWORTH GB, pages 279-281, XP002049519 see the whole document ---	1,2,9-14

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/17012

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 69, no. 5, 29 July 1968 Columbus, Ohio, US; abstract no. 19045q, N.MOHANTY ET AL: "New therapeutic agents of the quinoline series. I.Fused quinolyl compounds" page 1792; XP002049521 see abstract & J.INDIAN CHEM.SOC., vol. 44, no. 12, 1967, pages 1001-1004,	1-6, 24-26,29
X	CARLO BAEZNER: "Überführung von o-nitro- und o,p-dinitro-benzylchlorid in acridinderivate" BERICHTE DER DEUTSCHEN CHEMISCHEN GESELLSCHAFT., vol. 37, 1904, WEINHEIM DE, pages 3077-3083, XP002049520 see the whole document	1-4, 24-26
X	CHEMICAL ABSTRACTS, vol. 41, no. 20, 20 October 1947 Columbus, Ohio, US; abstract no. 6571bg, NGUYEN-HOAN ET AL: "Syntheses from o-halogenated anisoles and phenetoles" XP002049522 see abstract & COMPT.REND., vol. 224, 1947, pages 1228-1229,	1,2,9,10
Y	WO 92 21661 A (GLAXO INC) 10 December 1992 see claims	1-29
Y	EP 0 496 634 A (GLAXO INC) 29 July 1992 see claims	1-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/17012

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 15-23
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Inter. .onal Application No
PCT/US 97/17012

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9221661 A	10-12-92	US 5166159 A	24-11-92
		AU 2178792 A	08-01-93
		US 5318976 A	07-06-94
EP 0496634 A	29-07-92	US 5126351 A	30-06-92
		AU 1231492 A	27-08-92
		WO 9212981 A	06-08-92